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WATER TRANSPORT RATES OF THE TUNICATE *CIONA INTESTINALIS* [1]

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The interaction of suspension feeders with the environment depends primarily on the ability of these animals to transport sea water through their bodies. Although the determination of transport rates has been actively pursued by many investigators (Jørgensen, 1966), controversy exists over the accuracy and relevancy of the value produced by the use of a given method (Edmondson, 1966). The extreme rates, or the approximate average rate, are difficult to establish satisfactorily for tunicates due to experimental problems in handling the biological materials (*e.g.*, Hecht, 1916; Hoyle, 1953; Carlisle, 1966). Water transport rates of the tunicates *Styela clava* and *Ascidiella aspersa* were determined by different methods in static and in running sea water (Holmes, 1973). Even though the measured rates span a wide range, experiments with static sea water appear to yield lower transport rates than do those with running water. To establish sea water transport values for *Ciona intestinalis*, we are reporting transport rate studies using a variety of techniques. The average rates, or the extreme rates, are useful for determining the characteristics of sea water transport in suspension feeders and the relation of transport to tunicate physiology.

Materials

The holding tanks of the New England Aquarium provide abundant specimens of a local species of tunicate, *Ciona intestinalis*. The freshly filtered sea water in which the experimental materials were maintained had a temperature of 16.0 ± 0.2° C, an average salinity of 30.0 ± 0.5‰ (~0.51 M NaCl), and an average pH of 7.8 ± 0.3 at the time of experimentation. All specimens were kept in flow-through aquaria.

The 2-Methylquinoline (Quinaldine, Eastman Organic, practical grade) was used as anaesthetic to facilitate attachment of the tunicates to Pyrex tubes (≤ 6 mm O.D.) (Kustin, Ladd and McLeod, in preparation). The dye selected was a blue or green food color (Durkee's Food Color). The solvents for this dye, water and propylene glycol, were removed by freeze drying. The dye was then redissolved in sea water. At dye concentrations of approximately 0.01 ml original dye per 25.0 ml sea water, and/or high transport rates, *Ciona* was observed to react to the dye by squirting and ceasing to transport sea water.

In some experiments sea water-dye solution was supplied to the specimen with a Sage Variable Speed Tubing Pump, Model 375A. The Sage pump was calibrated by measuring the volume delivered to a clean dry graduated cylinder in a known time interval.

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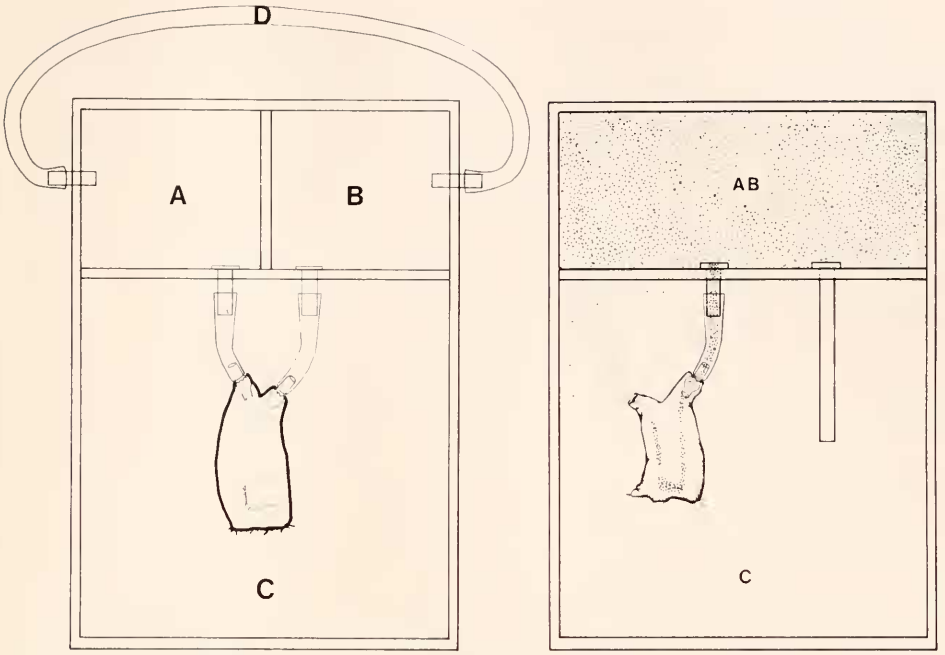

FIGURE 1. Overhead views of systems for determining the rate of sea water transport by the tunicate using the transfer of dyed sea water solution from compartment to compartment. The schematically rendered tunicate in the diagram shows the position of the attached specimen with respect to each compartment. *Ciona* which were used for experimental purposes lay horizontally, supported by the bottom of the container. *Left* shows *Ciona* attached by both siphons to Pyrex tubes with tygon tubing. Compartment A is attached to incurrent siphon; compartment B is attached to excurrent siphon; compartment C contains the body; tube D maintains equal levels. *Right* shows *Ciona* attached by incurrent siphon only to compartment AB. Compartment C contains the body. Plume of dye emitted by tunicate is schematically rendered. Return tube prevents the mixing of AB and C.

Dunaliella euchlora was grown at room temperature under fluorescent light banks in one gallon bottles in supplemented sea water type *f* (Guillard and Ryther, 1962). Stock cultures were similarly maintained sterilely at 19° C in 125 ml Erlenmeyer flasks. Algae were concentrated by centrifugation at 2500 rpm and resuspended in fresh sea water.

METHODS AND RESULTS

Volume methods

Specimens were attached to a partitioned chamber (FIG. 1, left, with connecting tube D absent). A typical rate of transport for a 4.0 g wet weight specimen was 180 ml hr^{-1} (or 45 ml/hr/g wet weight) determined by measuring the volume of overflow from Chamber B in 30 minutes. Careful observation showed that even under these conditions surface tension effects generated a pressure head approximately 1 mm between Chambers A and B.

Connecting tube D (Fig. 1, left) was added to eliminate the pressure head and a blue non-toxic dye was introduced into Chamber A. The rate was determined by monitoring the change in the absorbance of the solutions in Chambers A and B. One milliliter samples were removed simultaneously from the chambers at approximately six-hour intervals. Sea water was then carefully removed from Chamber C to keep all levels equal. Since the solution was pumped circularly the following treatment was used to calculate the flow rate produced by the tunicate.

In Chamber A, m_A is the moles of dye, C_A is the concentration of dye and V_A is the volume of sea water; similar terms, but with subscript B, refer to Chamber B. If the volume of solution transferred between chambers is ΔV, then the instantaneous rate of change of dye in Chamber A is

$$dm_A/dt = (d(\Delta V)/dt)\ (C_B - C_A) \tag{1}$$

assuming mixing in Chamber A. The total number of moles, M, of dye is conserved; therefore,

$$M = m_A + m_B \tag{2}$$

Also, the absorbance, A, of the solution in Chamber A is given by

$$A_A = alC_A \tag{3}$$

where a is the molar absorptivity coefficient, and l is the pathlength through the dye solution. Insertion of (2) and (3) into (1), rearrangement, and integration over the limits $m_A = m_A^0$ at time, $t = 0$ and $m_A = m_A$ at time, $t = t$ assuming constant flow so that $d(\Delta V)/dt = u$ = constant, and $V_A = V_B$ yields

$$ln\left(\frac{A_B - A_A}{A_B^0 - A_A^0}\right) = -2\left(\frac{u}{V_A}\right)t \tag{4}$$

The solutions in Chambers A and B were gently stirred to assure uniformity as infrequently as possible, since the tunicates often responded to stirring by diminishing transport. In a typical experiment, a 2.3 g specimen had an average rate of (5 ± 0.5) ml/hr/g wet weight, which appeared to be a rather low value.

Stress on the tunicate is relieved by reducing the number of attachments. Accordingly, only the incurrent siphon was connected (Fig. 1, right), the partition between Chambers A and B was removed, and the specimen transported sea water from Chamber AB into C. A return tube between AB and C kept the system at zero hydrostatic head. The samples for absorbance readings were taken approximately every 30 minutes for up to four hours, and analyzed according to equation (4). The results are presented in Table I.

In a further refinement, two specimens of *Ciona intestinalis* of approximately the same weight were attached to plastic bags partially filled with sea water. The bags were filled so as to exclude air bubbles, stoppered, weighed and allowed to equilibrate in a sea water bath. Under these conditions the pressure inside the bag should equal that outside. The specimens of *Ciona intestinalis* were anaesthetized and a Pyrex tube inserted in one siphon. They were then transferred to the sea water bath, allowed to revive and carefully connected to the bags so as not to

TABLE I

Transport rates for Ciona intestinalis *determined by volume flow. Column headings refer to the following: m, wet mass* Ciona; V_{AB}, *volume of sea water in compartment AB;* V_C, *volume of sea water in compartment C; u, transport rate;* V', $(V_C + V_{AB})/V_C V_{AB}$.

m	V_{AB}	V_C	u/V'	u	u/m
g	*ml*	*ml*	hr^{-1}	*ml* hr^{-1}	*ml* $hr^{-1}g^{-1}$
1.3	78	1035	0.138 ± 0.006	10.0 ± 0.4	7.7 ± 0.3
3.0*	260	890	0.32 ± 0.01	65 ± 2	21.7 ± 0.8
3.0*	260	890	0.742 ± 0.006	149 ± 1	49.8 ± 0.4
3.0*	260	890	0.418 ± 0.006	84 ± 1	28.0 ± 0.4
3.0**	260	890	0.67 ± 0.34	134 ± 73	45 ± 24
2.5	260	1000	0.262 ± 0.006	54 ± 1	21.6 ± 0.5
2.1	78	1035	0.108 ± 0.009	7.8 ± 0.7	3.7 ± 0.3
2.1	260	1000	0.047 ± 0.004	9.6 ± 0.8	4.6 ± 0.4

* Same *Ciona* measurements over different 2 hour time periods in same day.
** *Ciona* as in * but measurement over 4 hours on next day.

change the mass of the system. One *Ciona* was connected via the incurrent siphon, the other via the excurrent siphon. After six hours the bags were carefully detached, stoppered and weighed. In this experiment a 2.2 g specimen transported in 71 g of sea water in 6 hours and a 2.5 g specimen transported out 64 g in the same period, yielding an average of 5 ml/hr/g wet weight. Volume methods appear to give low rates. Possible causes could be cilia impairment due to insertion of the tubes and/or stress. Therefore, the clearance technique was also used (Fox, Sverdrup and Cunningham, 1937).

Clearance method

Specimens were fed cultures of the green marine flagellate algae *Dunaliella euchlora*. The average size of *Dunaliella* was $4.6 \pm 0.9 \times 10^{-4}$ cm as determined by microscopical sizing. Twenty-four hours prior to experimentation, the tunicates were placed in aerated beakers containing known volumes of sea water. A 0.1–1.0 ml sample of *Dunaliella*, cell concentration $1–5 \times 10^8$ cells ml^{-1}, was then added. Aeration provided stirring, which, in addition to the motion of the algae,

TABLE II

Effect of time on cell counts with mixing by aeration. Organism is Dunaliella euchlora *in 100 ml sea water. Column headings refer to the following: t, time; N, cell counts.*

t	N
min	*cell* $ml^{-1} \times 10^{-5}$
0	1.14 ± 0.05
20	1.3 ± 0.8
40	1.20 ± 0.03
80	1.15 ± 0.07
mean	1.20 ± 0.07

keeps their distribution uniform and counteracts the action of gravity. A control experiment, consisting of an aerated beaker containing only sea water and *Dunaliella,* was sampled and cell counts taken to see whether gravitationally induced sedimentation was a factor that had to be taken into account. The data shown in Table II show that a uniform distribution of cells is maintained, within experimental error.

Samples were removed for counting at periodic intervals over a span of two hours to determine the rate of cell removal. Cell counts were made on a Wild microscope at 10×40 power using an American Optical Neubauer hematocrit.

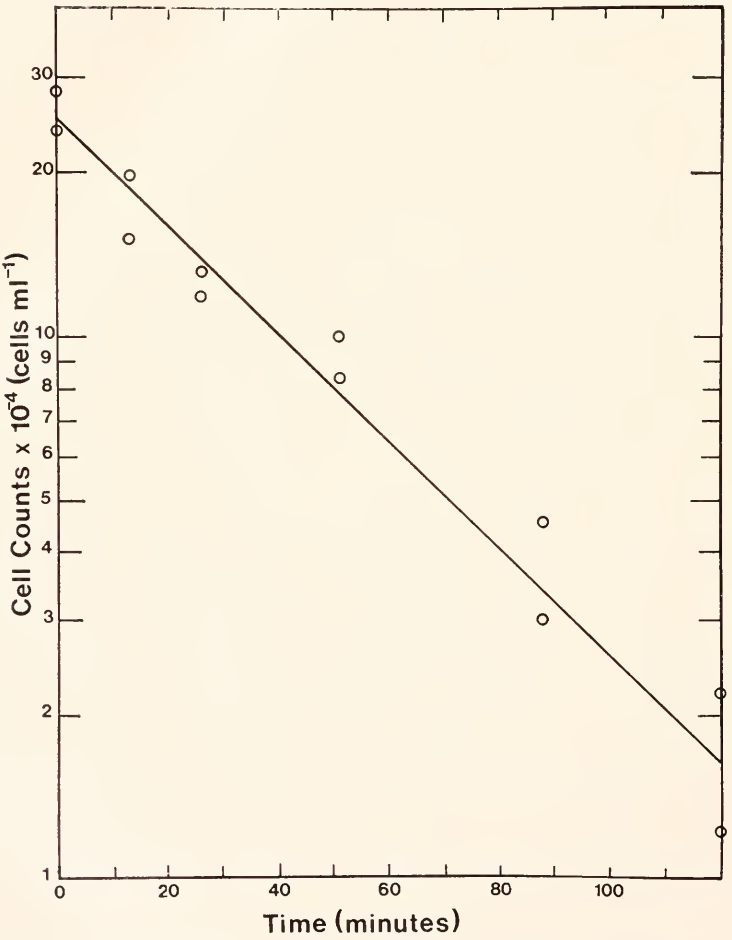

FIGURE 2. A semilog plot showing the relationship between cell counts per unit volume and time used in determining the rate of transport by particle clearance; experimental data: mass *Ciona,* 1.6 g; volume sea water, 100 ml; initial cell/ml $(2.47 \pm 0.04) \times 10^5$; u/V, 1.32 ± 0.12 hr^{-1}; u, 132 ± 12 ml/hr.

TABLE III

Transport rates for Ciona intestinalis *determined by clearance. Column headings refer to the following: m, wet mass* Ciona; *N, initial cell/ml; V, volume sea water; u, transport rate. Samples with same mass are same* Ciona *at different times.*

Day	m	N	V	u/V	u	u/m
	g	$cell\ ml^{-1} \times 10^{-5}$	ml	hr^{-1}	$ml\ hr^{-1}$	$ml\ hr^{-1}g^{-1}$
0	3.0	14.9 ± 0.8	250	2.2 ± 0.3	555 ± 70	185 ± 25
	1.5	13.7 ± 0.7	100	1.6 ± 0.2	160 ± 17	106 ± 18
	1.1	13.1 ± 0.4	100	1.02 ± 0.06	102 ± 6	92 ± 13
7	1.6	1.42 ± 0.04	100	1.3 ± 0.1	132 ± 12	82 ± 12
	1.6	2.47 ± 0.04	100	1.3 ± 0.1	132 ± 12	82 ± 12
	1.6	97 ± 3	100	0.82 ± 0.05	82 ± 5	51 ± 6
	2.4	2.5 ± 0.1	100	0.16 ± 0.01	16 ± 1	6.6 ± 0.7
	2.4	2.52 ± 0.06	100	0.11 ± 0.02	11 ± 2	5 ± 1
9	1.6	1.44 ± 0.07	100	0.11 ± 0.04	11 ± 4	7 ± 2
	1.6	23.2 ± 0.4	100	0.11 ± 0.02	11 ± 2	7 ± 1
	2.4	1.32 ± 0.09	100	0.78 ± 0.12	78 ± 12	32 ± 5
	2.4	22.5 ± 0.05	100	0.50 ± 0.04	50 ± 36	21 ± 15
	2.0	1.8 ± 0.1	100	0.28 ± 0.04	28 ± 6	14 ± 3
	2.0	25 ± 1	100	0.14 ± 0.04	14 ± 4	7 ± 2
15	1.6	10.9 ± 0.5	500	0.46 ± 0.10	231 ± 51	144 ± 32
	1.6	6.5 ± 0.2	500	0.40 ± 0.05	201 ± 27	125 ± 17
	2.4	10.7 ± 0.4	500	0.37 ± 0.04	183 ± 21	91 ± 10

The rate of removal was first-order (Fig. 2). The following first-order differential equation allows the transport rate to be calculated, assuming all cells passing into the tunicate are removed from the sea water.

$$-dC/dt = (u/V)C \tag{5}$$

In equation (5) C is cell concentration (cells/ml), u is the transport rate (ml/hr), and V is the volume (ml) of sea water in the beaker. The time constant u/V was determined by non-linear least squares analysis of equation (6) (Moore and Ziegler, 1960), the integrated form of equation (5), evaluated using the boundary condition $C = C_0$ at $t = 0$

$$C = C_0 e^{-(u/V)t} \tag{6}$$

Transport rates based on this assumption are at least minimum values, since not all particles may be removed. To determine if the system was affected by cell concentration or volumes in which the specimens were kept, experiments were performed at low and high cell concentration (and volumes) on the same specimen.

In only one experiment (1.4×10^6 cells ml^{-1}) was any effect of cell concentration noted. This experiment terminated when the tunicate ejected a large mass of cells in a mucus-like matrix in the same manner as that previously described (Jørgensen and Goldberg, 1953) with respect to graphite uptake. In our case, however, the ejection was from the excurrent siphon. Otherwise the results (Table III) were independent of cell concentration. In a similar fashion, the volumes of sea water in which the animals were kept did not affect the rate of transport.

TABLE IV

Transport rates for Ciona intestinalis *determined by direct methods. Values of u represent instantaneous maxima. Column headings refer to the following: m, wet mass of* Ciona; *u, transport rate.*

m	*u*	*u/m*
g	*ml hr*$^{-1}$	*ml hr*$^{-1}$*g*$^{-1}$
1.8	95	53
1.6	70	44
1.8	60–90	33–50
1.5	20	15
1.8	140	78
2.0	110	55
1.8	70	39
1.5	125	83
1.6	5	3
2.4	5	2
2.0	5	2.5
1.6	21	13

Direct method

A direct method (Hamwi and Haskins, 1969) was modified to permit monitoring of the flow of a dyed sea water solution through a tunicate positioned in an upright manner. A variable flow Sage pump presented the solution at a right angle to the orifice of the incurrent siphon at a uniform rate ($\pm 1\%$). The rate of flow of the dye solution was increased using the variable speed motor until it barely exceeded the rate at which the tunicate could remove it, whereupon the flow rate (assumed to be equal to that produced by the specimen) was recorded.

The specimens responded to the dye concentration. At high concentrations they would transport sea water until the solution reached the stomach; they then reacted by violently squirting out the dye. Consequently, the lowest possible dye concentration consistent with visibility ($A \sim 0.3$ at 635 nm) was used. With this technique, great variability in rates for a single specimen was noted. There were periods when the tunicates would not transport sea water; yet they appeared relaxed and normal. The values in Table IV are therefore instantaneous maxima and do not reflect prolonged transport.

DISCUSSION

Sea water transport in the tunicate serves the dual purposes of respiration and feeding. Neither of these functions requires continuous action. In fact, studies on the factors influencing rates of respiration have shown that, under stress, marine invertebrates may cease to transport sea water and respire anaerobically (Newell, 1973). Moreover, shutdown may follow exposure to low levels of oxygen (Holmes, 1973; Mangum and Van Winkle, 1973). Variability in transport has also been observed in our laboratory in connection with feeding and, of course, with squirting. Similar observations have been made for other ascidians; *e.g.*,

Phallusia mamillata C. (Hoyle, 1953). Thus, intermittant transport and variability in the rate of transport mean that no single flow velocity is characteristic of a given species. Nor should a given method be expected to produce a single rate value. The average value does not represent a true instantaneous rate, but is useful for a number of applications.

The maximum rate found with acceptable volume methods, namely 28 ml/hr/g wet weight, is less than the previously reported value of 80 ml/hr/g wet weight (Goldberg, McBlair and Taylor, 1951). We experienced difficulty in attaching *Ciona,* necessitating the use of the anaesthetic quinaldine. No mention of this problem is made in the previous report; indeed, the specimen is shown in a figure therein pumping out of the incurrent siphon. Perhaps a larger specimen was attached in the earlier study, with less impairment to the ciliary action. Our experience indicates, however, that the adventitious occurrence of pressure heads is rarely avoided in a "constant level" device such as that previously described (Goldberg, *et al.,* 1951). We conclude that the earlier value represents a hydrostatically assisted transport rate.

Clearance rate studies would appear to be a more reliable indicator of realistic rates, although this method also has drawbacks (Hamwi and Haskins, 1969; Holmes, 1973). The maximum value in the range of rates determined in this study, 185 ml/hr/g wet weight, is less than the previously reported value of 230 ml/hr/g wet weight (Jørgensen, 1966), although it is greater than that recorded in the volume method studies. The average value is 62 ml/hr/g wet weight. The range of values determined by the direct method overlaps with both the volume methods (lower limit) and clearance (the maximum directly determined value being 83 ml/hr/g wet weight). The average value of clearance and direct methods is 50 ml/hr/g wet weight, which represents a realistic reference value for computational purposes.

It is interesting to compare this result with studies on the rate of vanadium isotope exchange in the tunicate *Ciona intestinalis* (Kustin, *et al.,* in preparation). For, despite the intermittancy in transport and the variability in rate, the intrinsic time constant for vanadium assimilation from sea water is constant; *i.e.,* it is independent of tunicate mass, for individual tunicates or groups of experimental materials. Suspension feeding and respiration are subject to control, accomplished through a variation in the rate of sea water transport. Nevertheless, even at its lowest level, the lowest transport time constant is greater than the vanadium assimilation time constant. In terms of the average value we find 50 ml/hr/g wet weight to be much larger than 6.5×10^{-2} ml/hr/g wet weight (Kustin, *et al.,* in preparation). Hence, water transport is the more rapid of the two consecutive processes. Once the sea water source of vanadium is forced into the tunicate's body, an essentially chemical extraction process, subject to little or no control, takes place, and does not show variability.

In addition to the above application, another example of the use that can be made of the average water transport rate data, is to estimate the physiological demand this activity places on the organism. Our visual inspection of the dyed sea water as it passes through the specimen shows a smoothly flowing column of fluid, which suggests that the Stokes-Hagen-Poiseuille laminar flow equation might

be appropriate (Cole, 1962). This observation is supported by physiological studies of mucociliary action (Schlesinger, 1973), which suggest that the ciliary stroke produces a wave-like motion inducing smooth flow, and by a Reynolds number calculation on our data for *Ciona* (see below). By using fluid mechanics (Shames, 1962), the amount of mechanical, *i.e.*, pV (p is pressure), work done by the transport process can be related to the flow volume and the dimensions of the tube through which the volume of fluid flows.

We made a transparent photograph of *Ciona* transporting dyed sea water. The liquid column within the tunicate is visible through the translucent body. The photo was placed in an enlarger and brought to life size; the approximate dimensions of the column of dyed sea water were then measured. A 3.0 g specimen had dye solution in a U-shaped tube approximately 10 cm in length of narrowest diameter 0.08 cm and widest diameter 0.2 cm. (The plume of ejected fluid, clearly visible in the experimental aquarium and the photo, was approximately 0.15 cm wide and maintained this width for most of its length of 6–10 cm before dispersing). A diameter of 0.15 cm is therefore reasonable for the purpose of this estimation.

At an average rate of 50 ml/hr/g wet weight a 3.0 g specimen achieves a flow volume of 150 ml hr^{-1} or 4.2×10^{-2} cm^3 sec^{-1}. Sea water flowing at this rate in a 0.15 cm diameter pipe has a Reynolds number of $\sim$36 and would therefore exhibit viscous laminar flow (Shames, 1962).

The internal pressure generated by the ciliary "pump" is equal to the viscous loss in the tube through which the fluid passes. (The losses at the tube ends are negligible in comparison). The internal pressure, Δp, is given by

$$p = 128\, qL\mu/\pi D^4 \qquad (7)$$

where q is flow volume (4.2×10^{-2} cm^3 sec^{-1}), L is tube length exclusive of the "pump" (6.8 cm), μ is viscosity (1×10^{-2} dyne sec cm^{-2} for sea water at 20°), D is diameter (0.15 cm). The calculated internal pressure is 230 dyne cm^{-2} or $\sim$2 mm H_2O, which may be compared to values of 3–4 mm H_2O for sponges (Parker, 1914; Bidder, 1923), and 2 mm H_2O for *Ascidia atra* (Hecht, 1923). The energy lost at the end of the excurrent siphon (*i.e.*, at the end of the tube) is 2.8 dyne cm^{-2}, which is negligible compared with losses elsewhere. Flow volumes in excess of the maximum value reported here lead to significantly higher, physiologically unreasonable internal pressures based on viscous losses. Moreover, the value of $\Delta p \sim 2$ mm H_2O shows the considerable influence of even the smallest external pressure.

Summary

Sea water transport rates of the tunicate *Ciona intestinalis* were determined by measuring the volume of sea water transported through the specimen, and measuring the number of particles cleared by the specimen in a given time interval. The rate was also determined directly by matching the flow produced by the tunicate to that produced by a calibrated pump. *Ciona* transports sea water at variable rates; at times, it does not transport at all. The rate limits covering all techniques are: lower limit, 2.5 ml/hr/g wet weight and upper limit, 185 ml/hr/g wet

weight; the average value based on clearance and direct measurements is 50 ml/hr/g wet weight. Even at the lowest rate found, transport is rapid enough to ensure complete mixing between sea water and reaction or absorption sites in the pharyngeal chamber, alimentary tract or atrial chamber. We conclude that the rate controlling process for absorption of oxygen, vanadate ions, micro-organisms or organic detritus is not the rate of passage of the feeding current, but rather the rate of the intrinsic absorption process such as complex formation, ion exchange or adsorption.

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